Molecular characteristics of chronic hepatitis B virus infection among voluntary blood donors in Kinshasa, Democratic Republic of the Congo.

Pati Moloko Maindo^{1,2}, Email: patimaindo@gmail.com

Didier Anzenza Mudwahefa³, Email: didiermudwahefa@gmail.com

Jean-Pierre Kambala Mukendi¹, Email: jpmukkamb@gmail.com

Sylvain Ramazani Yuma⁴, Email: sylvainyuma@gmail.com

Jérémie Masidi Muwonga¹, Email: pmuwonga@hotmail.com

Gerald Misinzo^{2,5*}, Email: gerald.misinzo@sacids.org

Corresponding author: Gerald Misinzo²,

¹Department of Medical Biology, University Clinics of Kinshasa, Kinshasa, Democratic Republic of the Congo.

²Department of Veterinary Microbiology, Parasitology and Biotechnology, College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture, Morogoro, Tanzania.

³Department of Health Sciences, Environment and Sustainable Development, University of Versailles-Saint-Quentin-en-Yveline, Versailles, France.

⁴ National Blood Transfusion Centre, Kinshasa, Democratic Republic of the Congo.

⁵ SACIDS Africa Centre of Excellence for Infectious Diseases, SACIDS Foundation for One Health, Sokoine University of Agriculture, Morogoro, Tanzania.

21 Abstract

22 **Background:** Hepatitis B represents a major global health problem. Despite the high endemicity of
 23 hepatitis B in Sub-Saharan Africa, little is known about the molecular characteristics of chronic
 24 hepatitis B virus (HBV) infection in Africa, and there are very few published studies that describe
 25 the genetic characteristics of HBV in asymptomatic adults in DRC. The present study aimed at
 26 determining the molecular diversity of chronic HBV infection in voluntary blood donors in Kinshasa,
 27 DRC.

28 **Methods:** Blood samples from 582 voluntary blood donors at the National Blood Transfusion Centre
 29 in Kinshasa, DRC, were screened for hepatitis B surface antigen (HBsAg) using enzyme-linked
 30 immunosorbent assay (ELISA). Partial amplification and sequencing of S gene in HBV-positive
 31 samples was conducted.

32 **Results:** The presence of HBsAg was detected in 6.9 % (40/582) blood donors. Phylogenetic
 33 analysis based on partial S gene nucleotide sequences of HBV showed that the majority (66.7 %, 10/15) of HBV strains clustered into genotype A, followed by the genotypes E (26.6 %, 4/15) and D (6.7 %, 1/15). Genotype A strains were classified into subgenotype A1, quasi-subgenotype A3, and subgenotype A4, with quasi-subgenotype A3 being predominant. One new genotype A strain did not cluster with any existing HBV/A subgenotype or quasi-subgenotype.

38 **Conclusions:** The present study highlights the high genetic variability of chronic HBV infection in
 39 DRC, and the possibility of a new HBV/A subgenotype, suggesting that HBV has a long
 40 evolutionary history in DRC. Further molecular characterization of complete HBV genomes is
 41 needed for a more accurate assessment of HBV genetic variability and its clinical significance in
 42 DRC, as partial sequences are not appropriate for determining HBV new subgenotypes.

43 **Keywords:** blood donors, chronic hepatitis B, hepatitis B virus genotypes

44 Introduction

45 Hepatitis B virus (HBV) infection represents a major global health problem.
 46 According to the World Health Organization, an estimated 257 million people live
 47 with chronic hepatitis B infection [1], which is characterized by the persistence of
 48 hepatitis B surface antigen (HBsAg) in blood for a period of more than 6 months [2].
 49 About 15 to 40 % of HBV-infected patients develop cirrhosis, liver failure, or
 50 hepatocellular carcinoma (HCC) [3, 4]. In 2015, about 887 000 people died from
 51 end-stage liver disease or HCC [1]. The global prevalence of chronic HBV infection,
 52 defined as the prevalence of HBsAg, varies widely worldwide. Sub-saharan Africa
 53 have the highest rates, since more than 8 % of the population in these regions have
 54 chronic HBV infection [5].

55
 56 Owing to the high genetic variability of HBV, eight confirmed HBV genotypes
 57 designated A to H, two tentative genotypes named I and J, and around forty sub-
 58 genotypes have been described [6]. The HBV genotypes and subgenotypes are
 59 characterized by a minimum sequence divergence of 7.5 and 4 % of the entire
 60 genome, respectively [6-8]. Recently, some subgenotypes have been redefined into
 61 quasi-subgenotypes. Quasi-subgenotypes are lineages, which during full analysis
 62 taking into account all circulating strains of the same genotype and subgenotypes, do
 63 not fit the criteria of the subgenotypes [9]. Different HBV genotypes and
 64 subgenotypes/quasi-subgenotypes display differences in transmission routes,
 65 including sexual intercourse, unsafe injections, blood transfusions and mother-to-
 66 neonate transmission [6]. Moreover, many studies have shown that some genotypes

are associated with particular prognoses, such as progression to HCC, acute forms of hepatitis B or response to antiviral treatment. Thus, the knowledge of HBV genotypes and subgenotypes can help in predicting clinical outcome and planning suitable treatment [6, 10-13]. In addition, according to geographical distribution, HBV genotypes vary between continents, geographical regions, countries, and even between geographical regions within countries [12]. Genotypes A and E prevail in Africa, the former being mainly found in Eastern, Central and Southern Africa, and the latter being particularly restricted to West and Central Africa [13, 8]. Genotype D is predominant in Northern Africa [13].

Yet, data on the molecular characteristics of HBV in adults living in DRC are very limited. Some studies have been performed on very small sample sizes of adults [14-16]. Other studies included either symptomatic patients with jaundice from different provinces of the country [17] or adults living in the eastern part of DRC [18, 19]. The present study was performed in asymptomatic adults in Kinshasa, the capital city of DRC, located in the western part of the country.

Materials and methods

Study area, study design and sample size

The present cross-sectional study was conducted in Kinshasa, the capital city of DRC that lies on longitude 15° 19' 16'' East of Greenwich and latitude 4° 19' 19'' South

88 of Equator [20]. A total of 582 voluntary blood donors were enrolled in the present
89 study between November 2014 and December 2014.

90

91 **Study population and study exclusion criteria**

92 Blood samples from blood donors collected by the National Blood Transfusion
93 Centre (CNTS) during the study period were included in the present study. According
94 to the CNTS blood donation criteria, blood donors who had a history of transfusion
95 or jaundice, and all those belonging to a high-risk group, including drug addicts,
96 professional sex workers and those with multiple sexual partners, were excluded
97 from blood donation.

98

99 **Detection of HBsAg**

100 Serum samples were routinely screened for HBsAg using ELISA (Hepanostika®
101 HBs, Biomérieux, France and Abbott GmbH & Co. KG, Wiesbaden, Germany),
102 following manufacturer's instructions. Then, blood from HBsAg positive samples (50
103 µL) was drawn from either ethylene diamine tetra acetic acid (EDTA) vacutainer
104 tubes within 72 hours, or corresponding blood bags containing citrate phosphate
105 dextrose adenine (CPDA) anticoagulant within 7 days following blood donation, and
106 spotted into cards (FTA® classic cards Whatman® 3 mm). Afterwards, FTA classic
107 cards were dried and stored at room temperature until used for deoxyribonucleic acid
108 (DNA) detection and sequencing.

109 **Detection of HBV DNA**

110 FTA paper circles of 0.5 mm diameter containing blood samples were suspended in
 111 200 µL of lysis buffer and digested with 20 µL of proteinase K for 5 hours at 55 °C,
 112 followed by a standard nucleic acid extraction method using QIAamp genomic DNA
 113 Mini Kit (Qiagen Sciences, Maryland, USA), following the manufacturer's
 114 instructions. Then, HBV DNA detection was performed by amplification of the
 115 partial S-gene, using a nested polymerase chain reaction (PCR) that was expected to
 116 yield a product of approximately 400 base pairs. The following primer combinations
 117 were used: HBV_S1F (5' - CTA GGA CCC CTG CTC GTG TT - 3', nucleotide
 118 position 179) with HBV_S1R (5' - CG AAC CAC TGA ACA AAT GGC ACT - 3',
 119 nucleotide position 704) as outer primers, and HBV_SNF (5' - GTT GAC AAG AAT
 120 CCT CAC AAT ACC - 3', nucleotide position 217) and HBV_SNR (5'- GA GGC
 121 CCA CTC CCA TA -3', nucleotide position 658) as inner primers. These primers
 122 were previously used by Forbi *et al.* [21], with some modifications. After an initial
 123 denaturation step (10 min at 95 °C), DNA amplification was performed for 40 cycles
 124 at 95 °C for 30 sec, 55 °C for 45 sec and 72 °C for 45 sec, and a final extension at 72
 125 °C for 10 minutes using a Veriti 96-Well Thermal Cycler (Applied Biosystems,
 126 Jurong, Singapore). The first PCR round was performed with 2 µL of DNA and 1 U
 127 of *Taq* DNA polymerase (Invitrogen, Carlsbad, CA, USA) in a final volume of 20
 128 µL. The second PCR round was performed with 2 µL of DNA of the PCR product
 129 from the first round of amplification and 1 U of *Taq* DNA polymerase (Invitrogen,
 130 Carlsbad, CA, USA) in a final volume of 20 µL.

131

132 **Sequencing of PCR products**

133 A total of 5 μ L of PCR amplicons were loaded onto a 1.5 % GelRed-stained agarose
134 and the DNA bands were visualized using a gel documentation system (Gel Doc Ez
135 Imager, Bio-Rad, CA, USA). PCR products were denatured at 95 °C for 10 min in
136 the thermal cycler to avoid degradation by nucleases. Then, they were sealed and
137 stored at -40 °C until sequencing. The sequencing was carried out by Macrogen
138 Incorporation (Macrogen Europe, Amsterdam, The Netherlands) by using the
139 automated 3730xl DNA Analyzer machine and BigDye XTerminator Cycle
140 Sequencing Kit version 3.0 (Applied Biosystems, Foster City, CA, USA). HBV_SNF
141 and HBV_SNR were used as primers for sequencing of the partial S gene.

142

143 **Phylogenetic analyses**

144 The nucleotide sequences obtained were submitted to GenBank and were given the
145 accession numbers from KR535608 to KR535622 (Table S1). A similarity search at
146 GenBank nucleotide database was conducted using the Basic Local Alignment
147 Search Tool for nucleotides (BLASTn) [22]. The new HBV sequences from Kinshasa
148 along with 64 representative strains of genotypes A to H (Fig.1) were aligned using
149 Clustal W, and a phylogenetic tree was constructed using the Molecular Evolutionary
150 Genetics Analysis software (MEGA6.05) by the Maximum Likelihood method and
151 the Tamura three-parameter model of nucleotide substitution [23]. Bootstrap testing
152 of phylogeny was inferred following 1000 replications to assess the reliability of the
153 clusters. Values equal to or greater than 50 were indicated on the branches.

154

155 **Results**

156 In the present study, the presence of HBsAg was detected in blood donors, using
157 ELISA. A total of 582 blood donors including 503 men (86.4 %) and 79 (13.6 %)
158 women were enrolled. Among the 582 blood donors, 40 (6.9 %) were positive for
159 HBsAg. The median age of blood donors was 28 years (ranged from 18-64 years).
160 Out of the 40 HBsAg-positive samples, 36 were subjected to HBV-DNA extraction.
161 The four other samples could not be traced in the freezers after an initial HBsAg
162 screening. Hepatitis B virus DNA was also extracted from four samples used as
163 HBsAg positive controls by the CNTS. These positive controls were samples from
164 blood donors, which were strongly positive in HBsAg serological detection and were
165 then used as positive controls to subsequent ELISA. The detection of HBV DNA was
166 successful in 50% (18/36) of HBsAg positive samples and 2/4 of HBsAg positive
167 controls. From the 18 DNA samples, 14 were selected for sequencing, based on
168 quality of PCR products. Out of the 14 PCR products subjected to nucleotide
169 sequencing, 13 were successfully sequenced. Both DNA detected in the two positive
170 controls were also successfully sequenced. Thus, a total of 15 nucleotide sequences
171 were obtained and were named beginning with “DRC-Kin” followed by study
172 identification numbers or letters, e.g. DRC-Kin-9126519 or using their accession
173 numbers (Table S1). Ten (66.7 %) out of the 15 new nucleotide sequences clustered
174 into genotype A. Four new nucleotide sequences (26.6 %) clustered into genotype E.
175 One (6.7%) nucleotide sequence clustered into genotype D. The new HBV/A
176 sequences belonged to quasi-subgenotype A3 (5/10), subgenotype A4 (3/10) and
177 subgenotype A1 (1/10). Majority of the new HBV strains were closely related either

178 to strains from DRC alone, or to strains from DRC and other African countries. One
179 of the new HBV/A sequences (KR535612 DRC) did not cluster with any HBV/A
180 subgenotype (Fig. 1).

181

182 **Discussion**

183 The present study aimed at determining HBV genotypes among voluntary blood
184 donors in Kinshasa, the capital city of DRC, whose inhabitants originate from
185 different provinces of DRC. The high diversity of genotypes we found (genotypes A,
186 E, and D) is consistent with results reported by previous studies performed in
187 samples from subjects originating from different provinces of DRC [17] and in 6
188 samples from Kinshasa and the western part of DRC [14].

189

190 Majority of HBV strains found in the present study (66.7%) belonged to genotype A,
191 followed by genotype E (26.6%). Though Kinshasa is located in the western part of
192 DRC, our results seem to be contradictory to earlier studies reporting the
193 predominance of HBV/E in the western part of DRC [14] and in samples collected
194 from subjects having acute febrile jaundice and originating from different provinces
195 of DRC [17]. This discrepancy may be due to differences in study populations and/or
196 population sizes. Kinshasa is also likely to be an exception in the West-African
197 genotype E crescent, but this has to be confirmed by future studies performed in
198 larger sample sizes.

199

200 Seven HBV/A subgenotypes were described, namely HBV/A1, HBV/A2, HBV/A3,
201 HBV/A4, HBV/A5, HBV/A6 and HBV/A7 [2428, 2529]. But further, a new
202 classification was proposed, grouping together subgenotypes A3, A4, A5 and A7 into
203 “quasi-subgenotype A3” and renaming HBV/A6 into HBV/A4 [9, 2529]. The new
204 HBV/A strains we reported clustered into three subgenotypes, namely A1, A4 and
205 quasi-subgenotype A3, the latter being the most frequent HBV/A subgenotype. This
206 supports previous studies reporting HBV/A3 as predominant in West/Central Africa
207 [26] The particularity of HBV/A4 is to be a recent HBV/A subgenotype that has so
208 far been reported in very few Africa countries including DRC and two out of its
209 neighbouring countries, namely Republic of Congo and Rwanda [16, 27]. This high
210 HBV/A variability suggests that HBV has a long evolutionary history in Kinshasa,
211 DRC. One HBV/A new strain study did not cluster with any known HBV/A
212 subgenotype. The possibility that this strain be a new HBV/A subgenotype is not
213 excluded. Further molecular characterization of complete HBV nucleotide sequences
214 is needed in order to accurately assess the HBV/A genetic variability in DRC, as
215 results obtained from the partial sequence are not appropriate for introducing novel
216 HBV subgenotypes [9, 28].

217

218 **Conclusion**

219 The present study principally aimed at determining the HBV genotypes circulating in
220 asymptomatic adults in Kinshasa, DRC. Three HBV genotypes were found, namely
221 A, D and E, with a predominance of genotype A/ quasi-subgenotype A3. One
222 HBV/A strain clustered into none of the known HBV/A subgenotype. Further

223 molecular characterization of complete HBV nucleotide sequences is needed for a
224 more accurate assessing of HBV genetic variability and its clinical significance in
225 DRC.

226

227 **Additional files**

228 **Additional file 1: Table S1.** HBV strains sequenced in the present study. (DOC 13
229 kb)

230 **Abbreviations**

231	BLASTn	Basic Local Alignment Search Tool for nucleotides
232	CNTS	National Blood Transfusion Centre
233	CPDA	citrate phosphate dextrose adenine
234	DNA	deoxyribonucleic acid
235	DRC	Democratic Republic of the Congo
236	EDTA	ethylene diamine tetra acetic acid
237	ELISA	enzyme-linked immunosorbent assay
238	HBsAg	hepatitis B surface antigen
239	HBV	hepatitis B virus
240	HCC	hepatocellular carcinoma
241	MEGA	Molecular Evolutionary Genetics Analysis software
242	PCR	polymerase chain reaction

243 **Figure legend**

244 **Figure 1. Genetic relationship of HBV in blood donors of Kinshasa.** Maximum
 245 Likelihood method was used to infer phylogenetic relationship, with the Tamura 3-
 246 parameter nucleotide substitution model and bootstrapping of 1000 replicates as
 247 implemented in MEGA6.05 software. Relevant bootstrap values, using a cut-off
 248 value of 50 %, are shown. Sequences retrieved from GenBank are represented by
 249 their accession and country names. New HBV-DRC strains are preceded by a filled
 250 red triangle. The scale bar indicates the number of nucleotide substitutions per
 251 site.(PDF 14 kb)

252

253 **References**

- 254 1. World Health Organization. Hepatitis B. 2016.
 255 <http://www.who.int/mediacentre/factsheets/fs204/en/>. Accessed 7 July 2019.
- 256 2. World Health Organization. Guideline for the Prevention, Care and Treatment of
 257 persons with Chronic Hepatitis B Infection, Geneva: World Health Organization;
 258 2015.
- 259 3. Lok AS. Chronic hepatitis B. N Engl J Med. 2002;346:1682-1683.
- 260 4. Hou J, Liu Z, Gu F. Epidemiology and Prevention of Hepatitis B Virus Infection.
 261 Int J Med Sci. 2005;2:50-57.

- 262 5. Schweitzer A, Horn J, Mikolajczyk RT, Krause G, Ott JJ. Estimations of
263 worldwide prevalence of chronic hepatitis B virus infection: a systematic review of
264 data published between 1965 and 2013. *Lancet*. 2015;386:1546–1555.
- 265 6. Pourkarim MR, Amini-Bavil-Olyae S, Kurbanov F, Van Ranst M, Tacke F.
266 Molecular identification of hepatitis B virus genotypes/subgenotypes: Revised
267 classification hurdles and updated resolutions. *World J Gastroenterol*. 2014;20:7152-
268 7168.
- 269 7. Kramvis A, Arakawa K, Yu MC, Nogueira R, Stram DO, Kehinde MO.
270 Relationship of serological subtype, basic core promoter and precore mutations to
271 genotypes/subgenotypes of hepatitis B virus. *J Med Virol*. 2008;80:27-46.
- 272 8. Kurbanov F, Tanaka Y, Mizokami M. Geographical and genetic diversity of the
273 human hepatitis B virus. *Hepatol Res*. 2010;40:14-30.
- 274 9. Pourkarim MR, Amini-Bavil-Olyae S, Lemey P, Maes P, Ranst MV. Are
275 hepatitis B virus “subgenotypes” defined accurately? *J Clin Virol*. 2010; 47: 356-360.
- 276 10. Miyakawa Y, Mizokami M. Classifying hepatitis B virus genotypes.
277 *Intervirology*. 2003;46:329-338.
- 278 11. Tatematsu K, Tanaka Y, Kurbanov F, Sugauchi F, Mano S, Maeshiro T, et al. A
279 Genetic Variant of Hepatitis B Virus Divergent from Known Human and Ape
280 Genotypes Isolated from a Japanese Patient and Provisionally Assigned to New
281 Genotype J. *J Virol*. 2009;83(20):10538.
- 282 12. Sunbul M. Hepatitis B virus genotypes: Global distribution and clinical
283 importance. *World J Gastroenterol*. 2014;20:5427-5434.

- 284 13. Kramvis A, Kew MC. Epidemiology of hepatitis B virus in Africa, its genotypes
285 and clinical associations of genotypes. *Hepatol Res.* 2007;37:S9-S19.
- 286 14. Mulders MN, Venard V, Njayou M, Edorh AP, Bola Oyefolu AO, Kehinde MO,
287 et al. Low Genetic Diversity despite Hyperendemicity of Hepatitis B Virus Genotype
288 E throughout West Africa. *J Infect Dis.* 2004;190:400-408.
- 289 15. Hannoun C, Soenderstroem A, Norkrans G, Lindh M. Phylogeny of African
290 complete genomes reveals a West African genotype A subtype of hepatitis B virus
291 and relatedness between Somali and Asian A1 sequences. *J Gen Virol.* 2005;
292 86:2163-2167.
- 293 16. Pourkarim MR, Lemey P, Amini-Bavil-Olyaei S, Maes P, Ranst MV. Novel
294 hepatitis B virus subgenotype A6 in African-Belgian patients. *J Clin Virol.*
295 2010;47:93-96.
- 296 17. Makiala-Mandanda S, Le Gal F, Ngwaka-Matsung N, Ahuka-Mundeke S,
297 Onanga R, Bivigou-Mboumba B, et al. High prevalence and diversity of hepatitis
298 viruses in suspected cases of yellow fever in the Democratic Republic of Congo. *J*
299 *Clin Microbiol.* 2017;55:1299-1312.
- 300 18. Shindano TA, Horsmans Y, Kabamba BM. Genetic and phylogenetic
301 characterisation of hepatitis B virus in the eastern part of the Democratic Republic of
302 Congo. *J Med Virol.* 2017; doi: 10.1002/jmv.24837.
- 303 19. Goemine L, Sindano A, Horsmans Y, Kabamba Mukadi B. A43 Molecular
304 epidemiology of hepatitis B virus in South Kivu, an eastern province of the
305 Democratic Republic of Congo. *Virus Evolution.* 2018; doi:10.1093/ve/vey010.042

- 306 20. Kinshasa, République démocratique du Congo - Ville et Village du monde.
307 <https://fr.db-city.com/République-démocratique-du-Congo--Kinshasa>. Accessed 10
308 August 2019.
- 309 21. Forbi, JC, Vaughan G, Purdy MA, Campo DS, Xia G-I, Ganova-Raeva L, et al.
310 Epidemic History and Evolutionary Dynamics of Hepatitis B Virus Infection in Two
311 Remote Communities in Rural Nigeria. PLoS ONE. 2010;5(7):e11615.
- 312 22. Altschul SF, Madden TL, Schäffer AA, Zhang J, Zhang Z, Miller W, et al.
313 Gapped BLAST and PSIBLAST: a new generation of protein database search
314 programs. Nucleic Acids Res. 1997;25:3389-3402.
- 315 23. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. MEGA6: Molecular
316 Evolutionary Genetics Analysis Version 6.0. Mol Biol Evol. 2013;30(12):2725-2729.
- 317 24. Hübschen JM, Mbah PO, Forbi JC, Otegbayo JA, Olinger CM, Charpentier, et al.
318 Detection of a new subgenotype of Hepatitis B virus genotype A in Cameroon but
319 not in neighbouring Nigeria. Clin Microbiol Infect. 2011;17:88-94.
- 320 25. Pourkarim MR, Amini-Bavil-Olyae S, Lemey P, Maes P, Van Ranst M. HBV
321 subgenotype misclassification expands quasi-subgenotype A3. Clin Microbiol Infect.
322 2011;17:947-949.
- 323 26. Forbi JC, Ben-Ayed Y, Xia GL, Vaughan G, Drobeniuc J, Switzer WM, et al.
324 Disparate distribution of hepatitis B virus genotypes in four sub-Saharan African
325 countries. J Clin Virol. 2013;58(1):59-66.
- 326 27. Angounda BM, Ngouloubi GH, Dzia AB, Boumba LMA, Baha W, Moukassa D
327 et al. Molecular characterization of hepatitis B virus among chronic hepatitis B

328 patients from Pointe Noire, Republic of Congo. *Infectious Agents and Cancer*.
329 2016;11:51.

330 28. Shi W, Zhang Z, Ling C, Zheng W, Zhu C, Carr MJ, et al. Hepatitis B virus
331 subgenotyping: history, effects of recombination, misclassifications, and corrections.
332 *Infect Genet Evol*. 2013;16:355-361.

333

334 **Acknowledgments**

335 We appreciate the excellent technical assistance of Christopher Kasanga from
336 Sokoine University of Agriculture, Morogoro, Tanzania, Donatien Kayembe and
337 Mireille Nganga from the University Clinics of Kinshasa, Kinshasa, DRC, of Paul
338 Masiangi from the School of Public Health in Kinshasa, DRC, and of Eddy Sokolua
339 from the CNTS, Kinshasa, DRC.

340 **Funding**

341 This work was funded by the Intra-Africa, Caribbean and Pacific regions (Intra-ACP)
342 academic mobility scheme.

343 **Availability of data and materials**

344 The datasets generated and/or analysed during the current study are available in
345 GenBank with accession numbers KR535611-KR535622.

346 **Authors' contributions**

347 PMM and GM and conceived and designed the experiments. PMM, GM and JPKM
 348 performed the experiments. GM, PMM and DAM analyzed the data. GM, PMM,
 349 SRY and DAM contributed reagents/materials/analysis tools. GM, PMM and JPKM
 350 drafted the paper. All authors read and approved the final manuscript.

351 **Competing interests**

352 The authors declare that they have no competing interests.

353 **Consent for publication**

354 Not applicable.

355 **Ethics approval and consent to participate**

356 All blood donors were older than 18 years and provided informed consent to
 357 participate. The approval and ethical clearance to carry out this study was granted by
 358 the ethics review committee of the Public Health School of DRC
 359 (ESP/CE/027/2015).

0.01